STABILIZATION OF CLAY SOIL BY MICP USING UREOLYTIC / NON-UREOLYTIC BACTERIA

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***Abstract*—** Generally, Clay soil is an expansive and weak soil so there is a need for improvement after that only it is useful to withstand structure. In this paper Bacillus subtilis and Bacillus megaterium were used for clay soil stabilization. By using the MICP technique the properties of clay should be increased through microorganisms. By the process of bio- augmentation, the cementation reagent and bacteria were mixed to the clay soil with different proportions and the calcite precipitation is formed which makes particles bind together. As a result, there is an increment in process at a certain stage the values get reduced and there is no change when adding cementation reagent and bacteria after this limit. This can be identified through the test reports of CBR and UCC. It is a cheaper and low-cost material for stabilization raw materials are also easily available.

Keywords— Clay soil, MICP, CBR test, UCC, microorganism, cementation reagent, Bacillus subtilis, Bacillus megaterium.

I. INTRODUCTION

Stabilization of soil which alters the one or more properties on it to desire the requirements. In this process, ureolytic and non-ureolytic bacteria were used to produce urease enzymes to hydrolyze the urea into ammonium and carbonic acid. Calcium carbonate cement is precipitated due to calcium ions with the help of bacterial cells. This precipitation coats the soil particle form soil cement matrix, thus increases desired mechanical properties include strength and stiffness of soil matrix. Microorganisms are present in natural soil but some of them are harmful to humans but many types of this microorganism are very useful. From this, I got a clear idea to use microorganisms for clay soil stabilization, Bacillus subtilis and Bacillus megaterium were used for this project. MICP (Microbial Induced Calcite Precipitation) technique is used to mix the microorganisms into the soil sample with the help of bio-augmentation. Here I used liquid form of bacterial cells with cementation reagent which consists of urea, calcium chloride (cacl2), nutrient broth, and distilled water. This reagent are prepared with 0.25M, 0.5M, 0.75M,1M and the bacteria were added with soil sample of 2%,4%,6%,8% of curing periods of 7,14,21 and 28 days. From this, I have to found the accurate proportions for attaining the higher strength of the soil sample. The tests UCS and CBR were conducted for different proportions and then compared with Bacillus subtilis and Bacillus megaterium.

II. MATERIALS USED

Clay Soil is taken as the soil sample because in which the strength determination is easily identified. The microorganism of Bacillus subtilis and Bacillus megaterium is used as a stabilizing agent in which certain nutrients were mixed with the clay soil.

A. Clay soil

Clay soil consists of illite, kaolinite, montmorillonite in which the mineral montmorillonite increases, meaning the soil tends to have more shrinkage and swelling properties. This soil particle is so tiny it can be viewed in only an electron microscope.it exhibits slow permeability result in high water holding capacity.

Fig. 1 clay soil sample

B. Bacillus subtilis

It is a rod-shaped gram-positive bacteria and optimal growth temperature from 25°C-35°C. Its genomic structure contains five signal peptidase genes that are essential for the secretion of antibiotics. Endospores of B.subtilis can tolerate UV exposure and high temperatures. It is the best bacterial champion for secreted enzyme production and is widely used in the biotechnology sector.

Fig. 2 Bacillus subtilis in liquid form

C. *Bacillus megaterium*

It is a rod-shaped, gram-positive bacteria used for soil stabilization and it grows at a temperature of 3°C to 45°C with an optimum around 30°C. These cells occur in pairs and chains joined by polysaccharides on the cell walls. Its popularity has increased in biotechnology for protein production.

Fig. 3 Bacillus megaterium in liquid form

1) Cementation reagent

The chemicals were considered for making cementation reagent. 3g/Lt of nutrient broth is required for bacterial survival in soil. All the substances are mixed with the required amount of distilled water.

TABLE 1
CEMENTATION REAGENT

Substance	Amount	Amount	Amount	Amount
M	**0.25**	**0.5**	**0.75**	**1.00**
CO(NH2)2 (Urea)	16.00g	32.00g	48.00g	64.00g
CaCl2	26.75g	53.50g	80.25g	107g
Nutrient Broth	3.00mg	3.00mg	3.00mg	3.00mg

2) Bio-augmentation

Biological augmentation means adding bacterias to the soil along with the substrates. The mixed soil sample is compacted at maximum dry density with optimum moisture content. In this process, curing was adopted by using moist sand and wet gunny bags.

3) MICP

Microbial induced calcite precipitation is a technique after the process of adding microbes and cementation reagent to the soil it starts to react with it and forms calcite precipitation to hold the tiny soil grains together as a soil-cement matrix. After this formation the properties of soil are changed for this it is compared with the untreated soil sample.

4) Mixing

The soil sample is mixed manually and different proportions were mentioned in TABLE 2. According to the proportions of microbes, cementation reagent and clay soil are added and allowed for a curing period of 7,14,21,28 days. Then it is allowed for testing to determine its value.

TABLE 2
MIXING PROPORTION

Mix designation	Mix	
2:98	0.25M cementation reagent + 2% bacillus subtilis + 98% soil (BS1)	0.25M cementation reagent + 2% bacillus megaterium + 98% soil (BM1)
4:96	0.50M cementation reagent + 4% bacillus subtilis + 96% soil (BS2)	0.50M cementation reagent + 4% bacillus megaterium + 96% soil (BM2)
6:94	0.75M cementation reagent + 6% bacillus subtilis + 94% soil (BS3)	0.75M cementation reagent + 6% bacillus megaterium + 94% soil (BM3)
8:92	1.00M cementation reagent + 8% bacillus subtilis + 92% soil (BS4)	1.00M cementation reagent + 8% bacillus megaterium + 92% soil (BM4)

II. TEST AND RESULTS

A. Geotechnical properties

TABLE 3
PROPERTIES OF CLAY SOIL

Properties	Values
Colour	Brownish
Dry density	1400-1800 kg/m^3
Liquid limit %	25-60 %
Plastic limit %	15 -40 %
Soil classification	CL
Free swell index	40-150 %

B. Natural moisture content

TABLE 4
NATURAL MOISTURE CONTENT

Sample	1	2	3
W1	77.28	79.74	81.78
W2	271.88	318.94	462.99
W3	250.62	296.13	415.65
W	**12**	**11**	**14**

The soil sample has mean natural moisture content of 12%

C. Particle size distribution

Its shows different grain size of particles present in a soil sample in which coarse fraction of soil is separated by graded mesh. It is also called dry analysis.

1).Sieve analysis

TABLE 5
SIEVE ANALYSIS

Sieve (mm)	Soil retained (gm)	Cumulative retained (gm)	% Cumulative retained	% Passing
4.75	21.265	21.265	4.253009	95.74699
2.36	15.752	37.017	7.403415	92.59659
1.78	22.362	59.379	11.87582	88.12418
1.18	31.58	90.959	18.19184	81.80816
0.6	42.02	132.979	26.59585	73.40415
0.3	96.25	229.229	45.84589	54.15411
0.15	25.28	254.509	50.9019	49.0981
0.075	43.69	298.199	59.63992	40.36008

2). Hydrometer analysis

It is also called wet analysis in which soil particles below 75µ are used for this test. The different sizes of particles below 75µ are shown in the gradation curve as

TABLE 6
HYDROMETER ANALYSIS

Sieve size (mm)	% finer
0.0368	35.436
0.0266	33.418
0.0190	30.593
0.0135	29.786
0.0100	25.750
0.0071	20.907
0.0051	16.871
0.0037	15.256
0.0027	12.835
0.0019	10.211
0.0011	8.395

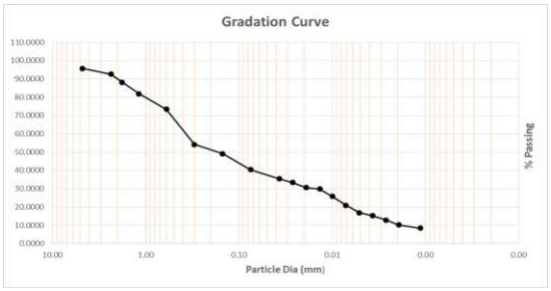

Fig. 4 Particle size distribution curve

D60 = 0.085 CU = 0.085 / 0.002 = 42.5
D30 = 0.011
D10 = 0.002 CC = $(0.011)^2$ / (0.002*0.085) = 0.71

D. Atterberg limit

i).Liquid limit

It is the limit in which soil tends to behave as liquid. The liquid limit of clay soil is obtained as 27 % by Casagrande method and the liquid limit comes out to be < 50%, therefore the clay soil is having low plasticity.

ii). Plastic limit

It is the limit in which soil Water paste changes form semi solid state to plastic consistency. The average plastic limit of clay soil was obtained as 13%.

iii). Plasticity index

Plastic Index (PI) = Liquid Limit(WLL) –Plastic Limit (WPL) = 27-13=14% .

E. Shrinkage limit

It is one which are mainly found in cohesive soil, there is a loss of water content in a soil which does not cause decrease in volume.

TABLE 7
SHRINKAGE LIMIT

Shrinkage dish	1	2	3
Initial water content of wet soil pat W1(g)	37.83	38.8	37.65
Mass of oven dry soil pat in gm Ws (g)	21.49	22.2	20.63
Volume of wet soil pat in cc (V1)	14.95	15	14.98
Volume of dry soil in cc (V2)	11.61	11.4	11.74
Shrinkage limit WSL (%)	11.98	10.8	11.96

The shrinkage of soil is found to be 11.58%

F. Specific gravity

TABLE 8
SPECIFIC GRAVITY

Sample	1	2	3
W1 = Weight of Empty Density bottle	492.42	492.42	492.42
W2 = Weight of Density bottle + oven dry soil	699	719	742
W3 = Weight of Density bottle + oven dry soil + water	1288	1301	1315
W4 = Weight of Density bottle + water full	1156	1156	1156
Specific gravity	2.77	2.78	2.76

The specific Gravity of soil is 2.77

G. Standard proctor compaction test

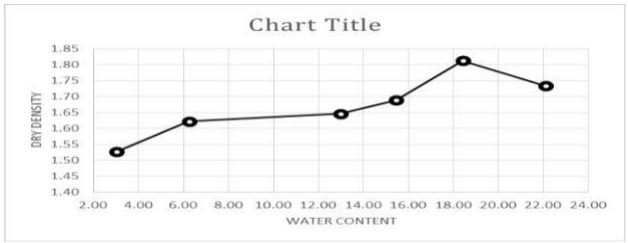

Fig. 5 proctor compaction

From this dry density and optimum moisture content was 1.81 g/cc and 18.42% beyond this is decrement in compaction process.

H. Unconfined compressive strength

UCC test were conducted for soil sample each proportions were compared in graphical representation.

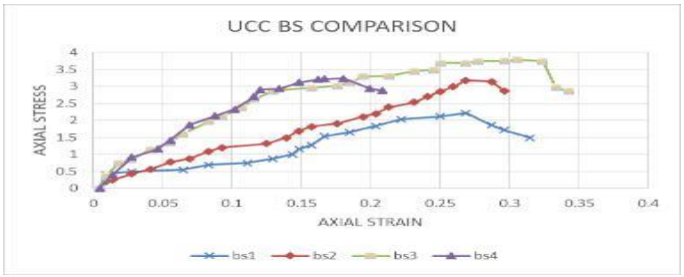

Fig. 6 UCC Bacillus subtilis

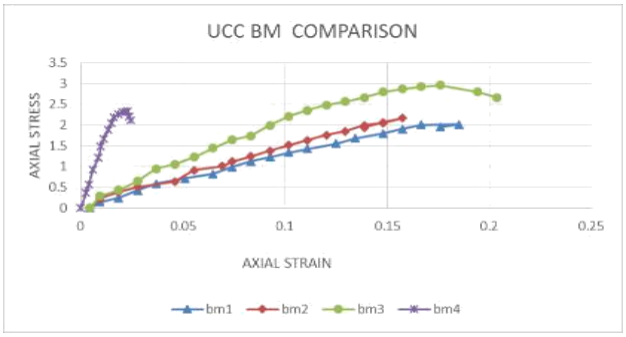

Fig. 7 UCC Bacillus megaterium

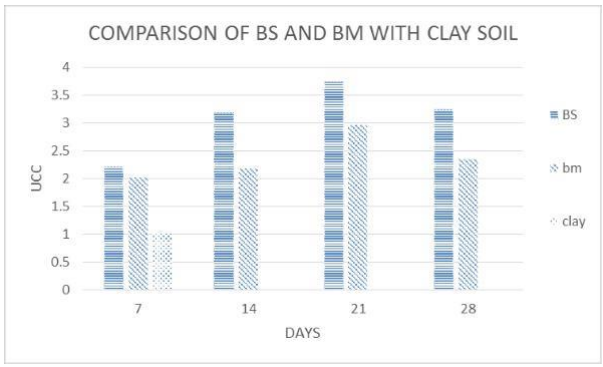

Fig. 8 UCC for Bacillus subtilis, Megaterium and clay soil

I. California bearing ratio

CBR test were conducted for soil sample of different proportions and this is shown in graphical representation as shown below,

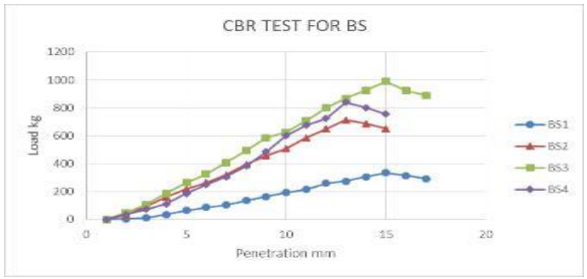

Fig. 9 CBR Bacillus subtilis

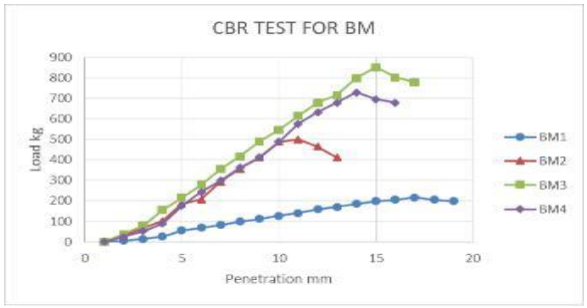

Fig. 10 CBR Bacillus megaterium

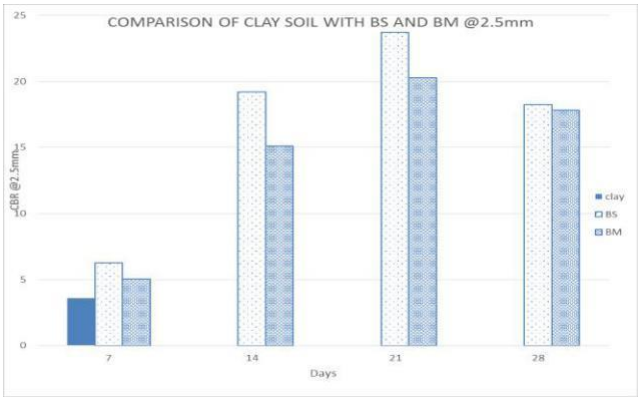

Fig. 11 CBR for 2.5 mm Bacillus subtilis, Megaterium and clay soil

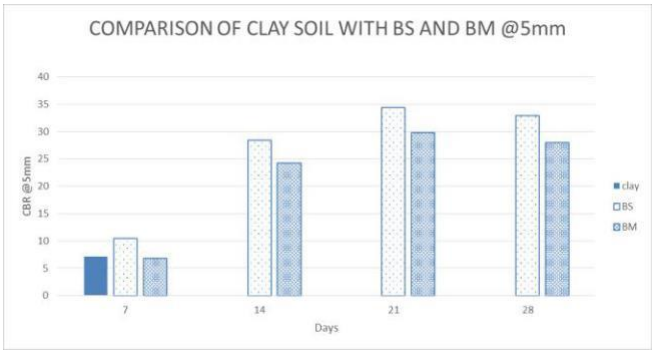

Fig. 12 CBR for 5 mm Bacillus subtilis, Megaterium and clay soil

IV. CONCLUSION

The soil sample should be compacted at obtained dry density and moisture content and the test were conducted shows that the values of CBR and UCC attained the peak strength of adding 6% of microbes, beyond this limit the strength is decreased in which bacillus subtilis shows maximum peak strength as compared to bacillus megaterium. From the results, I concluded adding microbes to soil is good stabilizing agent .The MICP is a cost effective technique and it can be prepared in huge amount at very low cost. Cementation reagent is also very economic as compared with other stabilizing technique. It is sustainable and eco-friendly which promises a great future.

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